Pathogenic Microbial Sensors for Coastal Safety

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LONG-TERM GOALS

The overall goal of this work involves the development of sensors for the detection of human pathogens in the coastal zone that may represent a risk to military personnel. A hand-held sensor is the immediate goal, while longer term goals may employ this technology on an autonomous platform such as an AUV.

OBJECTIVES

Our specific objectives are to optimize Nucleic Acid Sequence-based Amplification (NASBA) for NLV detection. In parallel, we plan to optimize sample collection and concentration technology. Finally, we will combine both together with hand-held sensor technology under development at the USF Center for Ocean Technology.

APPROACH

This report covers the first 4 months of this project. The first task was the hiring of a postdoctoral fellow to perform the research. Six candidates were interviewed at the American Society for Microbiology Meeting in Washington, DC in May, 2003. Stacey Patterson was offered the position, and she will start October 6, 2003 (she completed here PhD in the interim).

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14. ABSTRACT
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Our first task was to obtain Noroviruses from other labs and putative collaborators. We have established a collaboration with Christine Moe from Emory University in Atlanta. Dr. Moe provided us with a sample of the Genogroup I type of Norovirus. We also established a closer collaboration with Dr. Lilian Starke, of the Florida Department of Health labs near the University of South Florida in Tampa. She has provided us with a Group II Norovirus sample. Additionally, I am working with her graduate student, Matthew Smith, on primer design for Noroviruses. The second task was to develop Nucleic Acid Sequence-Based Amplification (NASBA) technology for amplification and detection of Noroviruses. NASBA is an isothermal method of RNA amplification based upon the action of three enzymes, AMV Reverse Transcriptase, T7 RNA polymerase, and RNAse H (Figure 1).

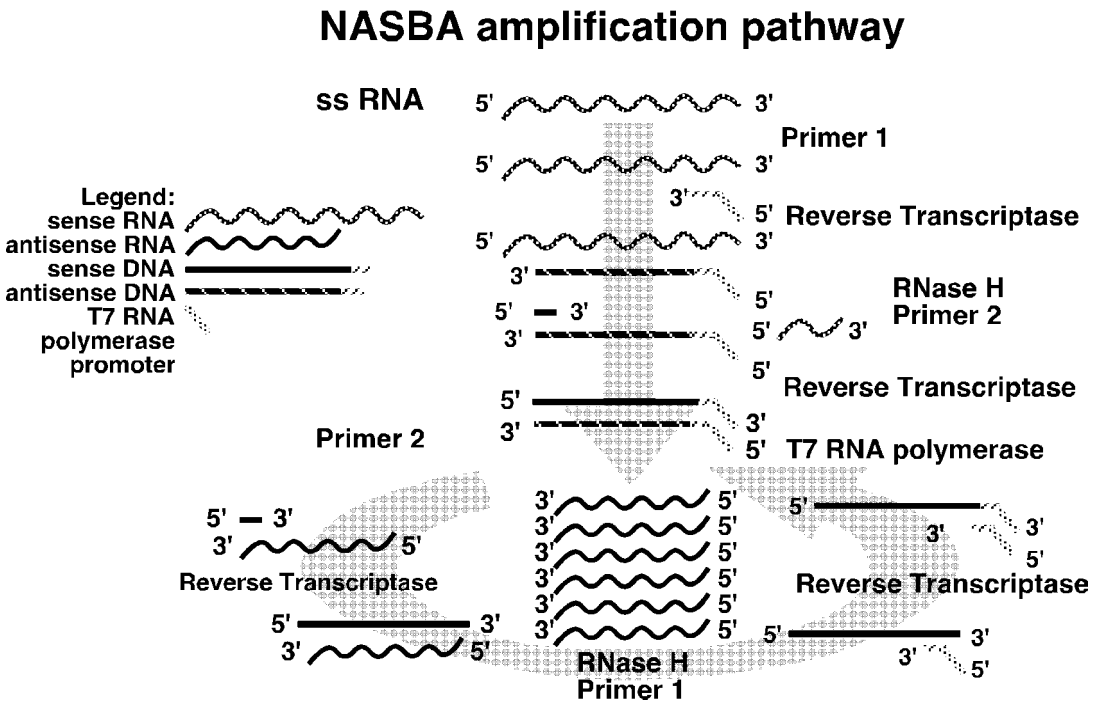

Figure 1. NASBA amplification pathway begins with a ssRNA target (in this case, the Noroviral genome), to which a primer (Primer 1) binds.

An RNA/DNA hybrid is made by the action of reverse transcriptase. RNaseH then degrades the RNA component of the hybrid, and reverse transcriptase using Primer 2 makes a cDNA of the target gene. Because Primer 1 contains the T7 RNA polymerase promoter, many copies of RNA are made, resulting in target amplification.

The preferred method of detection of target amplicons is the use of Molecular Beacons, which are hair-pin molecules that self-anneal (Figure 2). Each contains a fluor (6-carboxy fluorescein) on the 5' end and a quencher (DABCYL) on the 3' end. When self annealed, the fluorescence is quenched by the quencher. Upon binding to the target, the quencher is separated from the fluor, and the beacon fluoresces.

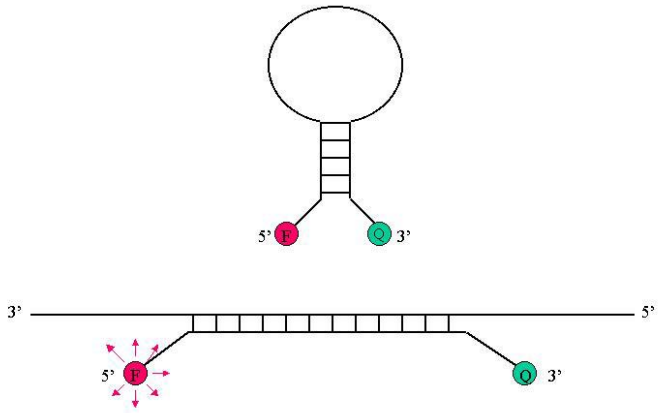

Figure 2. Molecular Beacon in the unbound hairpin conformation (upper figure) and in the fluorescent, target bound conformation.

We are in the process of designing NASBA primers and beacons for Norovirus Genogroup I and II. Additionally, with cost-sharing funds from the University of South Florida, we have recently purchased an EasyQ NASBA detection system from BioMerieux.

Figure 3. Nuclisens EasyQ Reader that detects NASBA amplification in Real Time.

We have had tremendous success with Enteroviruses as targets for NASBA. Figure 4 shows the results of an enteroviral amplification using Polio Sabin 1 virus and the EasyQ reader. 1000 poliovirus particles were amplified using two different NASBA reagent kits (multishot or single shot).

WORK COMPLETED

We have has tremendous success with Enteroviruses as targets for NASBA. Figure 4 show the results of an enterovirus amplified using Polio Sabin 1 virus and the EasyQ reader. 1000 poliovirus particles were amplified using two different NASBA reagent kits (multishot or single shot).

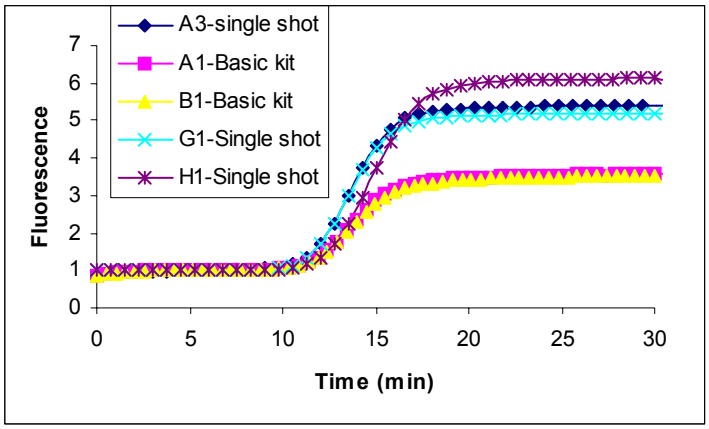

Figure 4. Results obtained with Nuclisens Basic Kit reagents or single shot NASBA reagents for detection of 1000 enteroviral particles.

We have used the bioinformatic software KODON to align both Norovirus Group I and Norovirus Group II genomes downloaded from GenBank. We have designed NASBA primer sets for both these groups as well as molecular beacons. We are in the process of evaluating these primers. We ran a denaturing agarose gel on our last Group II amplification, and it looks as if product formed (Figure 5). We have recently had success with the Genogroup !! primers.

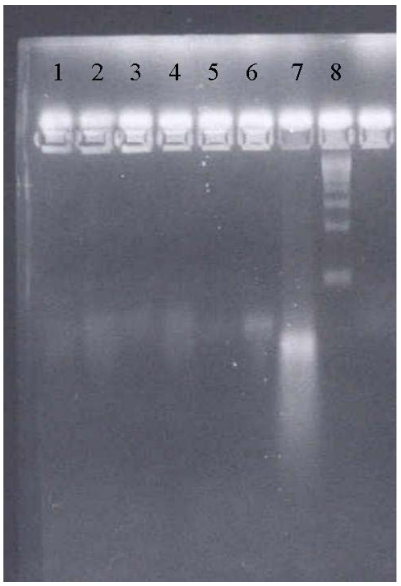

Figure 5. Denaturing RNA gel. Lanes 1,3, and 4 are NASBA attempts using Genogroup I primers. Lanes2, 5, and 6 are amplifications using Genogroup II primers. Lanes 1 and 2 are DI blanks. Lanes 3 and 5 are undiluted target (Norovirus Group I and II respectively). Lanes 4 and 6 are 1:10 diluted RNA extract (Norovirus Group I and II respectively). Lane 7 is successful amplification of the red tide organisms K. brevis. Of the Norovirus samples, Lane 6 (1:10 dilution of Group II) looks like it amplified.

Instrumentation considerations: We are in a dialogue with Tecan Group Ltd. which has designed an amplification platform in a CD-like format.
A visit was made to Tecan in Boston, MA to seek to insert their Lab on a CD technology into the project. A visit of their design and manufacturing facility was enacted. Negotiations are underway to transfer the technology into the project. The engineer that will support the integration of the CD is in preparation to come from Germany. Expected arrival is the second half of October.

The handheld system started under another project (Army contract) were this has progressed. The current configuration has a tube based optical reactor. The optical reactor, control program, circuit card controller are all-complete for the tube configured handheld. Next step is to package the handheld system together.

RESULTS

We have designed NASBA primers that work with Genogroup II Noroviruses, the most adundant forms of Noroviruses in the environment. This is the foundation for sensor development. Future work will be to investigate specificity of the NASBA primer, and capability to detect Genogroup I.

IMPACT/APPLICATIONS

This work represents the first successful development of a molecular beacon for Noroviruses. Adaptation of this technology to a hand-held sensor should result in several patents.

TRANSITIONS

This technology may have wide reaching application in the field of molecular diagnostics. It is hoped that this technology will be adapted to a series of different platforms in the future.

RELATED PROJECTS

We are involved in another (NSF Biocomplexity) project aimed at a field of molecular monitoring applications as part of a buoyed array monitoring system in Tampa Bay. This work is also related to another project developing an autonomous microbial genosensor.

In addition, we are also involved in an Army SMDC project whose purpose is to develop fieldable micro systems for detection chemicals and biologicals in the field. This project is benefiting from that project.